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# Membrane traffic as a coordinator of cell migration and junction remodeling

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**The change in the overall shape of developing organs is a consequence of the cumulative movement, reshaping and proliferation of the individual mural cells that make up the walls of these organs. Recent observations suggest that the shape and the position of endothelial cells (ECs) in growing blood vessels are highly dynamic, implying that these cells remodel their junctions extensively and do not preserve their initial relative positions. In order to determine the mechanisms that confer the dynamic behavior of mural ECs, we tracked the trafficking of a cell junction protein complex that consists of the RhoA-specific guanine exchange factor (GEF) Syx, the scaffold protein Mupp1, and the phospholipid binding protein Amot.[1] We found that RhoA co-trafficked with this complex on the same endocytic vesicles, and that its cellular activity pattern was determined by Rab13-dependent trafficking. The vesicles were targeted by a Rab13-associated protein complex to Tyr$^{1175}$-phosphorylated VEGFR2 at the leading edge of ECs migrating under a VEGF gradient. These results indicate that the dynamic behavior of ECs in sprouting vessels is conferred by using the same protein complex for the regulation of both cell junctions and cell motility. Together with previous studies that demonstrated regulation of Rac signaling by Rab5-dependent trafficking,[2] it appears now that membrane traffic is tightly coupled to the regulation of Rho GTPases, and consequently, to the regulation of the actin cytoskeleton, cell junctions and cell migration.**

Current concepts concerning the maintenance of cell junctions and the regulation of cell migration were deduced primarily from experiments performed in simplified systems. The most common format has been 2D, with several variants, such as gap closure. 2D assays yielded a trove of important insights into the regulatory molecular mechanisms of cell junctions and cell migration, but they do not simulate adequately the behavior of cells in vivo in a 3D ensemble. This is particularly relevant to the morphogenesis of tubular organs that are formed by a continuous sheet of epithelial or endothelial cells. Whereas intercellular junctions would seem to pose an obstacle to the movement of individual cells in 2D models of migration,[3] the preservation of junctions during the collective movement of the cells that comprise the walls of tubular organs is essential for their function. Even if the relative positions of the mural cells of such organs, i.e., the tip and stalk cells of a growing blood vessel, had remained constant, their junctions would have had to remodel in order to accommodate the change in the overall shape of the vessel. The relative position of mural cells is anything but constant, however. Observations in numerous organs and animal models revealed that mural cells reshuffle continuously in a seemingly stochastic manner during development.[4-7] These observations indicate a high degree of cell autonomy within the walls of growing tubular organs. Clearly, mural cells are not simply pulled forward by the tip cells during organ extension, but locomote under their own power. Hence, mural cells have

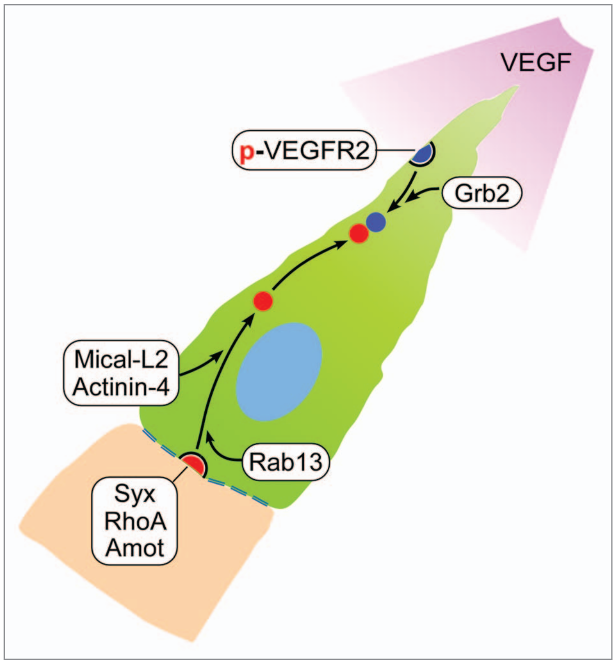


**Figure 1.** Proposed mechanism of Syx and RhoA trafficking from cell junctions to the leading edge. Vesicle trafficking from the junctions between endothelial cells of sprouting vessels is triggered by VEGF, and is mediated by Rab13. Mical-L2 and actinin-4 associate with vesicular Rab13 and target the vesicle to Grb2 bound to internalized autophosphorylated VEGFR2 that is located at the leading edge of the cell.

to multitask, i.e., they must remodel their junctions with neighboring cells while continuing to migrate. Though these cells do not have a free leading edge as the tip cells of the elongating vessel do, they move directionally and have to maintain, therefore, transient planar polarity. This highly dynamic behavior requires equally dynamic rearrangement of the cytoskeleton and its regulatory proteins within the cytoplasm of mural cells. In a recently published study,[1] we sought to elucidate the mechanisms that confer this dynamic behavior. We found that RhoA, a major regulator of the actin cytoskeleton, translocated from the junctions to the leading edge of ECs that migrated in response to a VEGF gradient. Surprisingly, the trafficking of RhoA was regulated by Rab13, a GTPase that was known primarily to mediate the recycling of tight junctions.[8] The RhoA-specific GEF Syx (named also Tech or GEF720) [9-11] co-trafficked with RhoA and activated it on the same vesicles (**Fig. 1**). When RhoA trafficking was impaired by silencing *rab13*, ECs lost their directional response to VEGF, and RhoA activity in these ECs was no longer polarized along the VEGF gradient, as in the control EC group. We determined that Syx was recruited to vesicles indirectly by angiomotin (Amot), a protein of high affinity to monophosphorylated phosphatidylinositols,[12] which binds the scaffold protein Mupp1. In turn, Mupp1 binds Syx,[13] thus coupling it to Amot. Similar to Rab13, Amot, Mupp1 and Syx are involved in the regulation of cell junctions[14,15] (and unpublished data), suggesting that junctional proteins may frequently be appropriated for cell migration. This is exactly, however, the functional versatility that is likely to be essential for conferring the dynamic behavior of ECs in growing vessels.

The tight coupling between membrane traffic and the function of Rho GTPases that was demonstrated by our study was first shown for Rac.[2] Moreover, the study of Palamidessi et al. (ibid.) found that Rac co-trafficked with Tiam1 and was activated by it on endocytic vesicles, analogous to our finding that RhoA co-traffics with Syx and is activated by it. Whereas we did not establish a direct role for Rab13 in the activation of RhoA, the study of Palamidessi et al. determined that Rac was activated by Rab5 via phosphatidylinositol-3-kinase, through a yet unknown mechanism. Since Rab13 and Rab5 are not known to share the same endosomes, it appears that RhoA and Rac are targeted independently of each other, and that their spatial activity patterns are regulated by different mechanisms. The known reciprocal relation between the activities of Rac and RhoA[16] suggests, however, that their trafficking pathways do intersect. In an attempt to determine how RhoA-associated vesicles were targeted to the leading edge of migrating ECs, we mined the interactome database and found that Grb2, an adaptor protein that binds phosphorylated tyrosines on several receptor tyrosine kinases (RTKs) including VEGFR2,[17] can associate with Rab13 via actinin-4 [18] and Mical-L2.[19] We then confirmed the association of Grb2 with Rab13 by immunoprecipitation. While we did not address the manner in which the Grb2-Rab13 complex is formed, the involvement of Grb2 in RTK endocytosis had been known.[20] Further, actinin-4 is a member of the CART recycling complex that includes myosin V,[21] an actin-based molecular motor that could propel the Rab13-associated vesicles. Recent studies showed that the endocytosis and trafficking of VEGFR2 are required for its function.[22-24] Collectively, these findings suggest that Rab13-associated vesicles are targeted to endosomes or vesicles that contain endocytosed VEGFR2. The manner in which these vesicles dock and possibly fuse with each other remains to be determined.

It has been increasingly recognized that the functions of membrane traffic are more versatile and coupled more tightly to cell signaling and migration than previously thought.[25-29] Rather than a specialized system solely for the mobilization of endocytosed receptors and ligands from the plasma membrane and back, endosomes have been recast as multifunctional platforms[25] and as a matrix deeply intertwined into the cellular architecture.[29] It is still unclear how membrane trafficking confers the specificity of individual signaling pathways. Several studies indicated that the endosomal menagerie is far more complex and transient than current conventional classifications, e.g., of a single static population of early endosomes.[30,31]

In the case of Rho GTPases, the specificity appears to be conferred at least in part by the identity of the associated Rab GTPase, i.e., Rab5 and Rab13 with Rac and RhoA signaling, respectively. Since Rab13, and to a larger extent, Rab5, are involved in numerous trafficking and signaling activities, it is very likely that additional factors are required for partitioning the sub-populations of Rab5 and Rab13-associated vesicles that regulate the activities of Rac and RhoA.

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